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EVOLUTION OF THE CATALYTIC FUNCTION OF THE GLUTAMATE OXIDASE SYSTEM

L. P. Zubok and Z. S. Kagan

Institute of Epidemiology and Microbiology imeni A.N. Gamalea, Academy of Medical Sciences USSR and Institute of Biochemistry imeni A.N. Bakh, Academy of Sciences USSR

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Measurements were made of the activation energy for oxidation of glutamic acid by the enzyme systems of Rickettsia prowazekii, by E. Coli, by healthy mouse tissues and by the tissues of mice infected with Rickettsia prowazekii. The results are discussed in terms of the evolution of the enzyme systems.

One of the basic features associated with the evolution of metabolism during the origin and development of life on Earth was the ever increasing step-by step disintegration of the chemical process into individual successive chemical reactions (ref.1). This in itself lowered the activation energy of the overall chemical process, i.e., it lowered the quantity of energy necessary to bring the molecules into a state where they were capable of reacting. Another very important path associated with the decrease in the activation energy, and consequently with the acceleration and facilitation of the chemical reaction, consisted of the origin of specific biological catalyzer-enzymes. Since, in the process of the origin and development of life, the catalytic function of enzymes also underwent continuous evolution, we can speak about the "quality of enzymes" (refs. 2 and 3)--i.e., about the amount by which the enzyme decreases the activation energy of the reaction which it catalyzes compared with the uncatalyzed spontaneous reaction, as the indicator of the evolutionary development of an organism which contains this enzyme. Apparently this evolution of the enzyme is associated not only with the evolution of the active center, but also with the evolution of the tertiary and quaternary structure of enzyme protein. Of course the evolution of the catalytic function of enzymes (from the inorganic "enzymes" of primary, more primitive, organisms to the more complex multicomponent metallo-flavo-proteids (ref.4)) consisted not only in a progressive lowering of the activation energy of reactions by them, but also in an increase of specialization, i.e., in the transition from group specificity with respect to the type of chemical bond to a strict specificity with respect to one substrate. /982*

The significance in the value of activation energy as an indicator of the enzyme "quality" appears quite large to us. Its determination for the same enzyme or for the enzyme system in representatives which are remote from each other in regard to the relationship of organism groups is very important from the point of view of comparative evolutionary biochemistry.

In this connection it appeared of interest to us to compare the activation energy of glutamate oxidase system of the causative organism of typhus Rickettsia prowazekii and of the host organism -- the mammal. As a means of additional control we utilized the nonpathogenic bacteria Escherichia coli. Rickettsia prowazekii, which occupy an intermediate position between viruses and bacteria (ref. 5), do not have fully developed metabolic cycles. However, Rickettsia have individual enzymes or even enzyme systems. Thus Rickettsia prowazekii (refs. 6 and 7)

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* Numbers given in margin indicate pagination in original foreign text.

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and Rickettsia mooseri (ref.8) actively oxidize glutamic acid with the formation of CO_2 and NH_3, i.e., they contain a glutamate oxidase system (glutamate dehydrogenase, a system for the oxidation of the α-ketoglutaric acid which is uninhibited by malonate (ref.9) and a cytochrome system (ref.10) for transfering electrons to O_2). Rickettsia also have the capacity to oxidize α-ketoglutaric, pyruvic, oxalacetic and succinic acids (ref.9), although at a much slower rate than glutamic acid. However for Rickettsia the oxidation of glutamic acid is /983 of the greater significance, because the presence of this acid in the agent is one of the basic conditions for the retention of biological properties by Rickettsia (ref.6) and because Rickettsial infection leads to a sharp increase in the oxidation of glutamic acid by the injured organs of white mice (refs. 7 and 9).

The oxidation of glutamic acid in washed suspensions of Rickettsia, grown in the yolk glands of a chicken embryo and isolated by differential centrifugation (ref. 11), as well as in the marrow homogenates of healthy mice and of mice infected with the Rickettsia prowazekii in liver homogenates of healthy mice and in suspensions of E. coli cells were determined in the Warburg apparatus (in an O_2 atmosphere) in Ringer-Krebs phosphate buffer (pH 7.2) at different temperatures. The E. coli were grown on meat-peptone agar. The cells were suspended and washed with the above buffer. Monosodium glutamate was used as the substrate. The infected animals were sacrificed in the agonal state. It was determined earlier by means of special experiments (ref.7) that the agonal state itself (produced by ebolism) does not change the oxidation rate of glutamic acid by homogenates of various tissues. The value of the activation energy was computed by means of the Arrhenius formula:

$$\mu = R \ln Q \frac{T_2 T_1}{T_2 - T_1},$$

where R is the gas constant (1.983), Q is the temperature coefficient, i.e., the ratio of reaction rates at absolute temperatures T_2 and T_1.

As we can see from Table 1, in spite of the fact that the washed suspension of the Rickettsia prowazekii a virulent strain which was grown in the yolk glands of a chicken embryo (the E strain) had a glutamate oxidase activity which was 1.2 - 1.5 times greater than that of the suspension of a virulent strain (the Brynl strain) grown under the same conditions, the values of the activation energy were very close and were 20,460 and 18,020 cal/mol respectively. It is interesting to note that the magnitude of the activation energy of the glutamate oxidase system in the Rickettsia prowazekii grown in the lungs of white mice was even greater and constituted 30,000 cal/mol (ref.6). Apparently the glutamate oxidase of system in the Rickettsia prowazekii, which were cultivated in the yolk glands of chicken embryos, is somewhat greater in a qualitative respect than the same enzymic system of Rickettsia isolated from the lungs of white mice.

TABLE 1. THE OXIDATION OF GLUTAMIC ACID BY A WASHED SUSPENSION OF VIRULENT AND AVIRULENT STRAINS OF RICKETTSIA PROWAZEKII AT DIFFERENT TEMPERATURES (ENDOGENOUS RESPIRATION WAS ABSENT).

Experiment number	N total of the suspension in mg.	Absorption of O_2 in microliters per hour		Q
		At 32°	At 37°	
	The Breynl Virulent Strain			
1	2.8	58	104	1.79
2	2.9	70	121	1.73
3	2.7	33	55	1.70
4	2.6	51	87	1.70
5	2.7	43	73	1.72
Average				1.73
Energy of activation 20,460 cal/mol				
	The Avirulent E Strain			
1	2.7	70	112	1.60
2	2.8	72	123	1.70
3	2.8	62	100	1.60
4	2.8	86	140	1.62
5	2.8	73	119	1.60
Average				1.62
Energy of activation 18,020 cal/mol				

Remark: The quantity of O_2 and N_{total} is given as calculated for 1.5 ml of suspension.

However, in itself, the absolute value of the activation energy for a particular enzymatic reaction cannot serve as the "quality" indicator of the catalytic properties of an enzyme. This quantity must be compared either with the activation energy of a spontaneous noncatalyzed reaction (i.e., we must know to what extent the enzyme decreased the activation energy), or with the activation energy for the same reaction which was catalyzed by the enzyme of a highly organized organism. In this connection we determined the value of the activation energy for the oxidation of glutamic acid, which was catalyzed by the homogenates of liver and marrow of healthy white mice and by the homogenates of marrow from white mice infected with the Rickettsia prowazekii. As we can see from table 2 the infection of mice leads to the activation of the glutamate oxidase system by a factor of 1.7, as noticed earlier (ref. 7,9). In this connection the value of the activation energy in both cases is equally low and constitutes 3830 cal/mol. This again confirms the conclusion made earlier (ref.7) that the increase in the oxidation of glutamic acid during

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TABLE 2. THE OXIDATION OF GLUTAMIC ACID BY LIVER AND MARROW HOMOGENATES FROM HEALTHY WHITE MICE AND BY MARROW HOMOGENATES FROM WHITE MICE INFECTED WITH THE RICKETTSIA PROWAZEKII AT DIFFERENT TEMPERATURES (ENDOGENOUS RESPIRATION IS EXCLUDED FROM THE DATA)

Experiment number	Marrow			Liver		
	Absorption of O_2 in microliters per 30 minutes		Q	Absorption of O_2 in microliters per 30 minutes		Q
	At 32°	At 38°		At 32°	At 38°	
			Healthy Mice			
1	27	31	1.13	65	75	1.15
2	28	32	1.14	60	70	1.16
3	26	29	1.11	75	90	1.20
4	29	32	1.13	65	77	1.18
5	27	31	1.12	80	97	1.20
Average			1.13			1.18
Activation energy 3827 cal/mol						
			Infected Mice			
1	43	50	1.14			
2	47	54	1.13			
3	47	53	1.13			
4	49	55	1.13			
5	49	54	1.10			
Average			1.13			
Activation energy 3827 cal/mol						

Remark: The quantity of O_2 is computed for 300 mg of tissue.

Rickettsia infection is associated with the activation of this enzymic system in the tissues of the host organism and not with the summation of the oxidase system of tissues belonging to the host and of cells belonging to the Rickettsia. The activation energy for the oxidation of glutamic acid by homogenates of liver from healthy mice turned out to be somewhat higher and was equal to 5183 cal/mol.

It follows from Table 3 that the activation energy of this same reaction which is catalyzed by the suspension of E. coli cells is also low and constitutes 6736 cal/mol, i.e., it is close to the value of the activation energy for the enzyme system from the liver of mice.

TABLE 3. THE OXIDATION OF GLUTAMIC ACID BY A WASHED SUSPENSION OF E. COLI CELLS AT DIFFERENT TEMPERATURES (DATA EXCLUDE THE ENDOGENOUS RESPIRATION)

Experiment number	Absorption of O_2 in microliters per 30 minutes		Q
	At 32°	At 38°	
1	229	285	1.24
2	222	289	1.30
3	230	291	1.26
4	236	283	1.20
5	229	290	1.22
Average			1.24

Activation energy 6736 cal/mol

Remark: The quantity of O_2 is given for 1.5 ml of the suspension (1.05 mg of N_{total}).

Thus the activation energy for the oxidation of glutamic acid in the Rickettsia prowazekii is 5-9 times greater than the activation energy of the same reaction which is catalyzed by the enzymic system of the mammal and the bacterium. This proves the primitive nature of the glutamate oxidase system in the Rickettsia cell. It is known (ref. 12) that the activation energy of biological systems is determined by an element which "sets the tempo", i.e., by the slowest process in the reaction chain. Taking into account the more ancient origin of anaerobic elements of metabolism compared with aerobic (ref. 1,13), we can assume that in the glutamate oxidase system of the Rickettsia the glutamate dehydrogenase which carries out the first reaction for the oxidation of glutamic acid is the most primitive. The primitive nature of the glutamate oxidase system for the Rickettsia cell places Rickettsia in close dependence on the combined reactions which take place in the host organism and explains the failure of efforts to develop an artificial medium for growing Rickettsia outside an organism.

Taking into account the irreversibility of the evolution process (ref.14) it is necessary to assume that Rickettsia are a "cul-de-sac" branch of evolution, i.e., they are initially parasitic organisms. It is known that parasitism in higher organisms (for example, in worms (ref. 15) and in phanerogamous plants (ref. 16)), is secondary and is accompanied by the depletion of certain enzymes or enzymic systems without a change in the remaining enzymes (ref. 17). In the light of data which we have obtained it is hardly proper to consider (ref. 18) the Rickettsia as common secondary parasites which occur in the usual manner during the course of evolution, i.e., after the origin of the host and not parallel with it, and to place Rickettsia in the evolutionary staircase between viruses and chlamydozoa.

REFERENCES

1. Oparin, A. I. Vozniknoveniye zhizni na Zemle (The Origin of Life on the Earth) Izdatel'stvo Akademii Nauk SSSR (Izd. AN SSSR), 1957.
2. Blagoveschenskiy, A. V. Uspekhi Sovremennoy Biologii, 11, 320, 1939.
3. Biokhimich, osnovy evolyutsionnogo protsessa u rasteniy (The Biochemical Principles of the Evolutionary Process in Plants) Izd. AN SSSR, 1950.
4. Goffman-Ostengof, O. V sborn: Vozniknoveniye zhizni na Zemle (IN: The Origin of Life on the Earth) Izd. AN SSSR, p. 126, 1957.
5. Zdrodovskiy, P. F. and Golinevick Ye. N. Ucheniye o rikketsiyakh i rikketsiozakh (Teachings on Rickettsia and Rickettsiosis) Moscow, 1956.
6. Zubok, L. P. and Blagoveshchenskiy, A. V. Biokhimiya, 22,695, 1957.
7. Zubok, L. P. Biokhimiya, 24, 745, 1959.
8. Balayeva, N. M., Zubok, L. P. Zhurn. Mikrobiol., epidemiol. i immunol., No. 8, 14, 1957.
9. Zubok, L. P. Biokhimiya, 28, 265, 1963.
10. Hays, J. E., Hanh F. E et al. Biochim et biophys acta, 26, 570, 1957.
11. Wisseman, C. L., Jackson, E. B. et al. J. Immunol 62, 123 1951.
12. Neylands, Zhd., Shtumf, P. Ocherki po khimii fermentov (Remarks on the Chemistry of Enzymes) I. L., 1958.
13. Oparin, A. I. Tr. V Mezhdunarodn. biokhim. Kongressa, Simpozium 3 (Transactions of the 5th International Congress on Biochemistry) Simpozium, 3, Izd. AN SSSR, p. 74, 1962.
14. Severtsov, A. N. Etyudy po teorii evolyutsii (Studies on the Theory of Evolution) Berlin, 1921.
15. Byuding E. Tr. V Mezhdunarodn. biokhim. kongressa, Simposiyum 3 (Transactions of the 5th International Congress on Biochemistry, Symposium 3) Izd. AN SSSR, p.280, 1962.
16. Bereznegovskaya, L. N. Zhurn. Obshch. biol., 24, 195, 1963.
17. Kretovich, V. L. Yevstigneyeva, Z. G. and others. V sborn. Problemy evolyutsionnoy i tekhnich. biokhimii. K 70-letiyu akad. A.I. Oparina (In Collected Works Problems of Evolution and Engineering Biochemistry. Dedicated to the 70th Anniversary of Academy Member A. I. Oparin) Nauka, p. 193, 1964.
18. Terskikh, I. I. Vestnik Akademii Meditsinskikh Nauk SSSR, No. 3, 67, 1964.

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